Molecular Biology of the Ewing's Sarcoma/Primitive Neuroectodermal Tumor Family

By Enrique de Alava and William L. Gerald

Abstract: **Ewing's sarcoma (ES) and primitive neuroectodermal tumor (PNET) are members of a tumor family consistently associated with chromosomal translocation and functional fusion of the *EWS* gene to any of several structurally related transcription factor genes. Similar gene fusion events occur in other mesenchymal and hematopoietic tumors and are tumor-specific. The resulting novel transcription factor–like chimeric proteins are believed to contribute to tumor biology by aberrant regulation of gene expression altering critical controls of cell proliferation and differentiation. These tumor-specific molecular rearrangements are useful for primary diagnosis, may provide prognostic information, and present potential therapeutic targets. The recent advances in our understanding of the molecular biology of ES and PNET represent a paradigm for the application of the basic biology of neoplasia to clinical management of patients.**

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EWING'S SARCOMA (ES) and primitive neuroectodermal tumor (PNET) were described in the early part of this century as distinct clinicopathologic entities. Arthur Purdy Stout[1] described a tumor of the ulnar nerve with the gross features of a sarcoma but composed of small round cells focally arranged as rosettes (subsequently designated peripheral PNET). James Ewing[2] described an undifferentiated tumor in the diaphysis of long bones that was radiosensitive (ES). In ensuing years, the overlapping characteristics of these two categories of small round-cell tumor have become well known, supporting the concept of a single tumor category with variable phenotypic expression and leading to the acceptance of a unified diagnostic grouping. The ES/PNET family also includes several other related clinicopathologic neoplastic entities, such as malignant small-cell tumor of the thoracopulmonary region (Askin's tumor), paravertebral small-cell tumor, atypical ES, PNET of bone, and extraosseous ES.[3-7] These tumors are poorly differentiated, small, round-cell tumors and often pose difficult diagnostic problems when examined by light microscopy alone. For that reason, they have become a model system for nonmorphologic approaches to diagnosis and subclassification, such as immunophenotypic and genetic analysis. From this multimodal investigation have come improved diagnostic criteria and a better understanding of their basic biology. In addition to sharing some clinical and morphologic features, these malignant neoplasms have common genetic, in vitro, and immunophenotypic characteristics that further validate the concept of a single, genetically defined tumor category.[8] Advances in our understanding of the basic biology of the ES/PNET family have had a direct impact on clinical care and places this tumor family at the forefront of intensive efforts to understand the relationship between genotype, tumor biology, and clinical phenotype.

CLINICAL AND PATHOLOGIC FEATURES

ES and PNET affect primarily white and Hispanic young people and is extremely rare in individuals of African or Asian origin. The reason for this striking ethnic distribution is not known, although interethnic differences exist for certain alleles of one of the genes consistently disrupted in ES and PNET.[9] Tumors can develop in almost any bone and soft tissue and often present with pain and swelling. Approximately 25% of patients have detectable metastatic disease to lung, bone, and bone marrow at diagnosis, but nearly all patients have micrometastases, as evidenced by a 10% cure rate with local therapy alone. The standard of care is systemic therapy combined with surgery or radiotherapy for local control. Despite advances in therapy, 5-year survival rates are only approximately 50%. The most important negative prognostic factor is tumor dissemination at the time of diagnosis.[10]

Typical histologic features include sheets of monomorphic round cells with small hyperchromatic nuclei, incon-

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spicuous nuclei, and scant cytoplasm. There is usually extensive necrosis with peritheliomatous preservation of viable tumor and a lack of intercellular material. Variants include larger cells, greater pleomorphism, more condensed chromatin, and a lobular or organoid growth pattern. Tumors can exhibit a variable degree of neural differentiation, but this is usually subtle and often only detected immunophenotypically. The histogenesis of ES and PNET is unknown; however, a neuroectodermal origin has been proposed based on variable expression of neuronal immunohistochemical markers, ultrastructural features, and the ability of ES/PNET cell lines to differentiate along a neural pathway in vitro.[11] Nevertheless, other histogenetic possibilities cannot be excluded because ES and PNET can also exhibit some epithelial and mesenchymal characteristics and can arise in organs not directly related to the neural crest (eg, the kidney).

No routinely used histochemical or immunohistochemical stains can positively distinguish ES and PNET from other undifferentiated tumors of childhood; however, in the vast majority of cases, ES and PNET have been shown to express at extremely high levels an antigen determined by the *MIC2* gene.[12,13] The product of *MIC2* is a glycoprotein (also designated CD99 or p30/32^{MIC2}) with a molecular mass of approximately 30,000 daltons located on the cell surface and believed to be involved in cell adhesion. Although immunohistochemical detection of membrane-localized *MIC2* expression is a sensitive diagnostic marker for the ES/PNET family of tumors, it lacks specificity in that many other tumors, and for that matter, many normal tissues, are also immunoreactive with anti-MIC2 antibodies in some cases (Table 1).[14] The diagnosis of ES and PNET has been largely a process of exclusion. In recent years, detection and investigation of specific genetic alterations have established exquisitely sensitive and specific markers for ES and PNET that have rapidly become the standard for confirming the diagnosis.

Table 1. Normal and Neoplastic Tissues That Can Be Immunoreactive With Anti-MIC2 Antibodies

Normal tissues
Some lymphocytes
Some columnar epithelia
Pancreatic islets
Renal collecting ducts and distal convoluted tubules
Urothelium
Vaginal squamous epithelium
Sertoli cells
Granulosa cells
Fibroblasts (variable)
Endothelium (variable)
Neoplastic tissues
Ewing's sarcoma and PNET
Leiomyosarcoma
Malignant fibrous histiocytoma
Chondrosarcoma
Fibrosarcoma
Thymoma
Lymphoma
Schwannoma
Astrocytoma
Neuroendocrine tumors
Rhabdomyosarcoma
Desmoplastic small round-cell tumor
Embryonal carcinoma
Bladder carcinoma
Esophageal carcinoma
Glioblastoma
Ependymoma
Wilms' tumor
Clear-cell sarcoma of the kidney
Uterine stromal sarcoma

MOLECULAR GENETICS OF ES AND PNET

Consistent Chromosomal Translocations in ES and PNET

An important advance in the study of mesenchymal and hematopoietic tumors has been the identification of consistent chromosomal translocations associated with unique tumor types (Table 2).[15] These translocations interrupt specific genes and recombine them to create novel fusion genes. The fusion genes are expressed and encode proteins that combine functional domains usually found in separate molecules. Because the fusion gene and its products are tumor-specific and present in virtually all cases of an individual tumor category, their characterization not only yields profound insights into tumor biology but also holds great promise for new diagnostic and therapeutic approaches.

Approximately 85% of tumors diagnosed as ES or PNET harbor the translocation t(11;22)(q24;q12) (Fig 1A).[16,17] At the molecular genetic level, the chromosome 22q12 breakpoints are clustered within a single gene designated *EWS* (for ES) and the chromosome 11q24 breakpoints are within a gene called *FLI1* (a gene homologous to the Friend leukemia virus integration site 1).[18,19] In this rearrangement, the distal portion of *FLI1* is juxtaposed to the proximal portion of *EWS*, thereby creating a functional *EWS-FLI1* fusion gene (Fig 1B). The encoded protein contains the amino-terminal domain of EWS and the carboxy-terminal region of FLI1, creating a completely new protein with a unique function. In the remaining ES/PNET, other chromosomal translocations are present that result in an analogous fusion of *EWS* to genes with structural homology to *FLI1*. These genetic changes are now considered a specific diagnostic feature of these tumors, and the fusion gene products

Table 2. Gene Fusions in Mesenchymal Tumors

Tumor Type	Translocation	Gene Fusion	Incidence (%)
ES/PNET	t(11;22)(q24;q12)	*EWS-FLI1*	85
ES/PNET	t(21;22)(q22;q12)	*EWS-ERG*	10
ES/PNET	t(7;22)(p22;q12)	*EWS-ETV1*	Rare
ES/PNET	t(17;22)(q12;q12)	*EWS-E1AF*	Rare
ES/PNET	t(2;22)(q33;q12)	*EWS-FEV*	Rare
DSRCT	t(11;22)(p13;q12)	*EWS-WT1*	95
Myxoid liposarcoma	t(12;16)(q13;p11)	*TLS-CHOP*	95
Myxoid liposarcoma	t(12;22)(q13;q12)	*EWS-CHOP*	5
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	*EWS-CHN*	75
Malignant melanoma of soft parts	t(12;22)(q13;q12)	*EWS-ATF1*	NK
Synovial sarcoma	t(X;18)(p11.23;q11)	*SYT-SSX1*	65
Synovial sarcoma	t(X;18)(p11.21;q11)	*SYT-SSX2*	35
Alveolar RMS	t(2;13)(q35;q14)	*PAX3-FKHR*	75
Alveolar RMS	t(1;13)(p36;q14)	*PAX7-FKHR*	10
DFSP	t(17;22)(q22;q13)	*COL1A1-PDGFB*	NK
Congenital fibrosarcoma and mesoblastic nephroma	t(12;15)(p13;q25)	*ETV6-NTRK3*	NK

Abbreviations: DFSP, dermatofibrosarcoma protuberans; DSRCT, desmoplastic small round-cell tumor; NK, not known; RMS, rhabdomyosarcoma.

are believed to play an important role in ES/PNET development and biology.

The EWS *Gene*

The *EWS* gene was identified as the site disrupted by the chromosomal translocation t(11;22)(q24;q12) found in ES and PNET. It encodes a widely expressed 656-amino acid protein of unknown function. The amino-terminal domain, that portion preserved in the ES/PNET fusion protein, is composed almost exclusively of a repeated and degenerated polypeptide motif having the consensus NSYGQQS. This domain shares distant homology to eukaryotic RNA polymerase II. The carboxy terminus, which is replaced by tumor-specific translocations, contains an RNA recognition motif.[20] EWS is a member of a growing family of highly conserved RNA-binding proteins, including TLS/FUS (from a gene translocated in myxoid liposarcoma and acute myelocytic leukemia), hTAFII68 (the TATA-binding protein–associated factor 68), the small nuclear ribonucleoprotein–associated 69 kd protein, the bovine Pigpen protein, and *Drosophilia* Cabeza/sarcoma-associated RNA-binding fly homolog.[21-28] These proteins share distinct structural characteristics in their carboxy termini (such as a ribonucleoprotein motif, arginine-glycine-glycine boxes, and a putative zinc-finger domain) that are believed to mediate interaction with RNA or single-stranded DNA.[29] Although the exact biologic functions of wild-type EWS and its relatives remain largely unknown, a growing body of evidence suggests that they are involved in mRNA transcription. Specifically EWS, hTAFII68, and TLS/FUS have recently been shown to localize within the TFIID complex, a multiprotein complex that participates in preinitiation assembly of the transcription apparatus on actively expressing genes.[24,30] EWS has been shown to associate with protein subunits and transcription products of RNA polymerase II and form a ternary complex with other heterogeneous RNA-binding proteins.[31] This suggests that EWS may play an important role in basic transcriptional regulation.

Translocation Partners of EWS *in ES and PNET*

The *FLI1* gene on 11q24 was the first *EWS* translocation partner identified in the ES/PNET family of tumors. It is a member of a large family of DNA-binding transcription factors that are implicated in the control of cellular proliferation, development, and tumorigenesis.[32-34] The family members are defined by the presence of a highly conserved 85-amino acid domain termed the erythroblastosis virus–transforming sequence (ETS) domain. This domain mediates specific binding to purine-rich DNA sequences characterized by a GGA(A/T) core element.[35] In ES/PNET-associated translocations, the ETS domain can be contributed from any of several family members. In 85% of cases, it is derived from *FLI1* [t(11;22)(q24;q12)], and in 10% of cases, it is from *ERG* (*ets*-related gene; t(21;22)(q22;12)).[36,37] In rare instances, *EWS* is fused to the ETS domains of *ETV-1* (*ets* translocation variant 1; t(7;22)(q22;12)), *E1AF* (E1A factor; t(17;22)(q12;q12)), or *FEV* (fifth Ewing variant; t(2;22)(q33;q12)).[38-40] The functional consequences of these less frequent variant translocations are thought to be analogous to the *EWS-FLI1* rearrangement, but subtle differences are likely to exist.

ETS domain–containing proteins act as either transcriptional activators or repressors. These functions are modu-

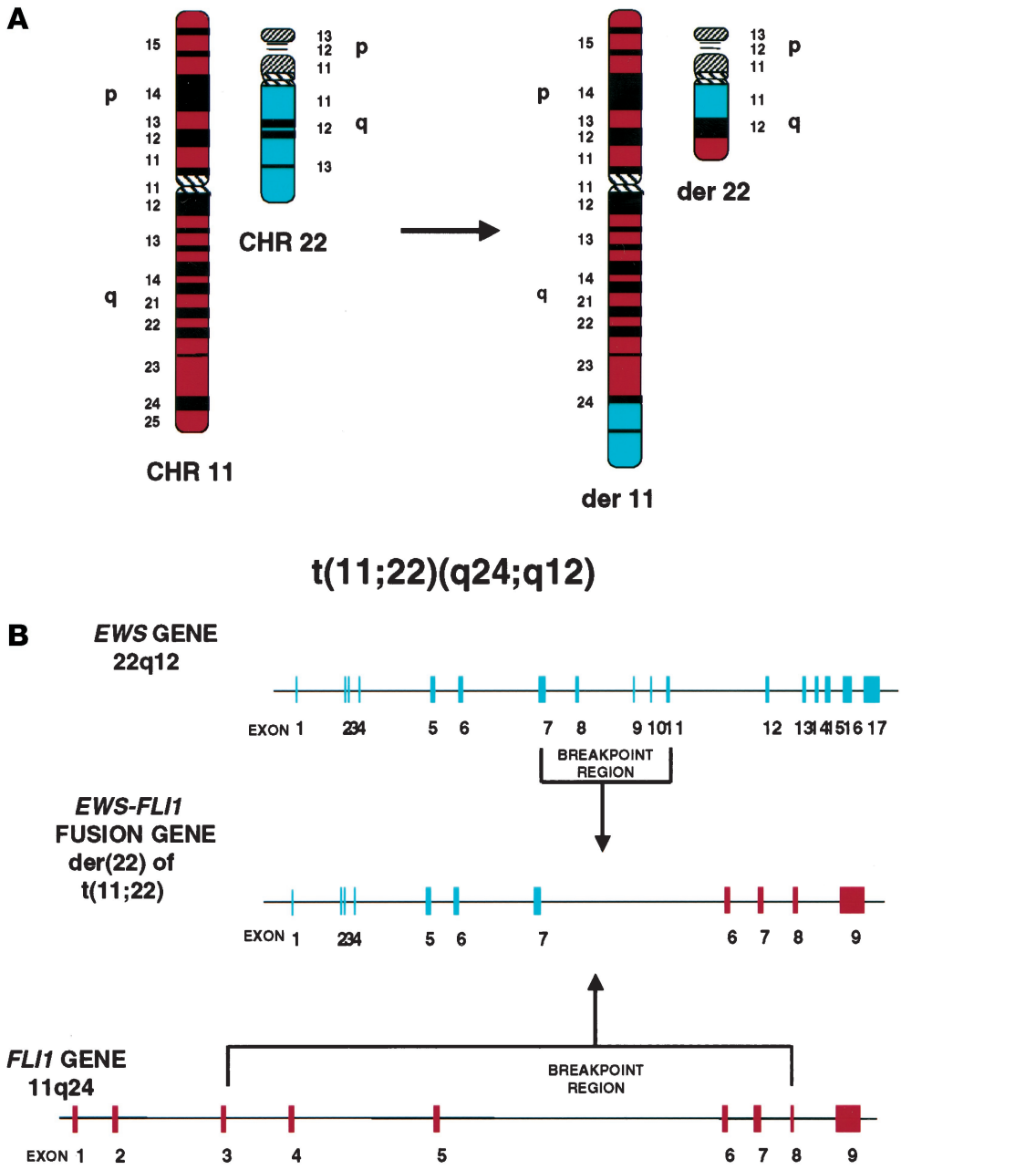

Fig 1. (A) Schematic diagram of t(11;22)(q24;q12) consistently associated with ES/PNET. (B) Schematic diagram of *EWS-FLI1* gene fusion resulting from t(11;22). Individual gene exons are depicted as numbered boxes, and the line represents intervening and flanking DNA. The regions where chromosomal breakpoints occur are bracketed.

lated by a combination of specific DNA-protein and protein-protein interactions. ETS family members have been shown to cooperate with other nuclear proteins and often function as part of a larger protein complex that helps to establish promoter specificity, modulates transcriptional regulation, and allows linkage to signal transduction pathways.[32-34] Many ETS domain proteins are targets of signal transduction pathways and are activated in response to a wide array of extracellular stimuli. For example, activation of RAS leads to mitogen-activated protein kinase–mediated phosphorylation of some ETS proteins.[41] Because RAS is implicated in cell-cycle regulation and tumor angiogenesis

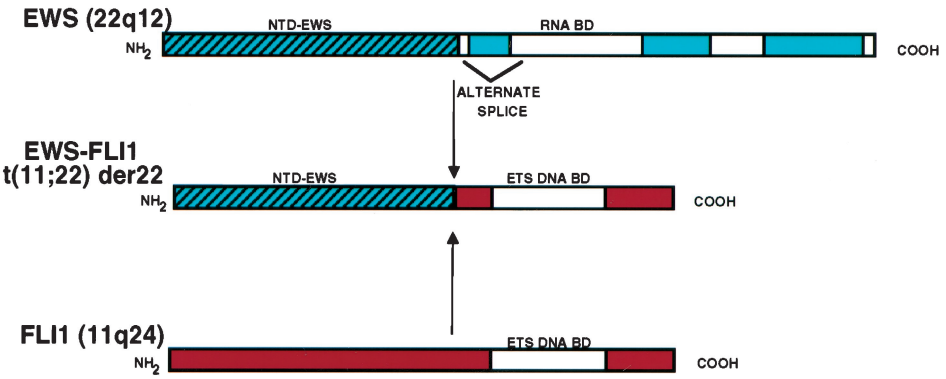

Fig 2. Schematic diagram of the chimeric EWS-FLI1 protein product (NTD, amino-terminal domain; BD, binding domain).

and is permanently activated in a high percentage of human cancers, it may be that RAS-responsive ETS proteins play a general role in oncogenesis.

ETS domain proteins are implicated in tumors other than ES and PNET.[34] Chromosomal translocation and fusion of the ETS domain of ERG to TLS/FUS is present in myelocytic leukemia, and fusion of the carboxy- or amino-terminal region of TEL to a variety of translocation partners occurs in some myelocytic and lymphoblastic leukemias. The *TEL* translocation partners include *AML1*, *ABL*, *PDGFR*-beta, *JAK2*, and *MN1*. The amino-terminal region of TEL does not contain the ETS DNA-binding domain but has a motif that mediates protein-protein interactions. The oncogenic role of TEL amino-terminal region–containing fusion proteins seems to be mediated by homotypic oligomerization and activation of gene products involved in growth-related pathways. These two different functional domains present in ETS proteins can contribute to tumorigenesis through unique mechanisms.

Translocation Partners of EWS *in Other Tumor Types*

Although translocations involving the *EWS* gene and ETS family genes only occur consistently in ES and PNET, fusions of *EWS* to different transcription factor genes have been observed in other tumors (Table 1). *EWS* is fused to *ATF1* in malignant melanoma of soft parts, *WT1* in intra-abdominal desmoplastic small round-cell tumor, *CHOP* in myxoid liposarcoma, and *CHN* in myxoid chrondrosarcoma.[42-46] In addition, an *EWS*-like gene, *TLS/FUS*, is involved in tumor-associated gene fusions in myxoid liposarcoma (*TLS/FUS-CHOP*) and acute myeloid leukemia (*TLS/FUS-ERG*).[21,22,47,48] Chimeric proteins in this group of tumors have a structure analogous to that of EWS-FLI1 described above and presumably a similar function. All result in the fusion of the amino-terminal portions of EWS or TLS/FUS to DNA-binding domains of transcription factors, further suggesting that disruption of transcriptional control contributes to their transforming potential. It is important to realize that in each case, with few exceptions, a distinct clinicopathologic tumor entity has been found to be consistently associated with a translocation involving a unique class of transcription factor. This tumor specificity is most likely related to cell-specific influences on DNA recombination, chimeric gene expression, and intrinsic proteins that are necessary to complement chimeric protein action.

Other Genetic Alterations in the ES/PNET Family

The consistent *EWS*-ETS gene family rearrangements present in the ES/PNET family indicate that they are a primary and probably rate-limiting step in tumorigenesis; however, other genetic alterations occur and may contribute to tumor biology. By conventional and molecular cytogenetics, frequent gains of chromosomes 8 and 12 and losses of 1p and an unbalanced der16t(1;16) have been identified.[49,50] These regions may contain genes that play a role in ES/PNET progression. In addition, changes in several known tumor-associated genes occur in some cases. Homozygous deletion of *CDKN2A* (*P16*, *INK4*) has recently been described in 18% to 30% of primary cases, and mutations of *p53* are detected in 5% to 20%.[51-53] Mutations affecting these two genes are more frequent in ES/PNET cell lines, indicating a selection bias for growth in culture.

CHIMERIC MOLECULE FUNCTION

Regulation of Transcription

The ES/PNET-associated translocations result in chimeric products containing the amino-terminal domain of EWS fused to the nucleic acid–binding domain of the transcription factor translocation partner (Fig 2). This structure suggests that the chimeric protein is directed to the promoter region of specific genes recognized by the translocated DNA-binding domain and contributes to tumor development by altering expression of these genes. In support of this, some *EWS* fusion products have been shown

experimentally to function as transcriptional activators.[54-57] The EWS-FLI1 protein localizes to the nucleus and can bind DNA in a site-specific manner via the ETS domain. The DNA-binding site specificity and affinity of EWS-FLI1 are similar to those of native FLI1, which suggests that the chimeric protein might interact with the same genes normally regulated by FLI1. However, the targets of action are not solely dependent on the ETS domain and may be affected by protein-protein interactions that are unique to the chimeric molecule and result in a different spectrum of targets.[58] EWS-FLI1 has been shown to be an efficient transcriptional activator when cotransfected with model reporter gene constructs containing ETS-binding sites in their promoters. This transcriptional activation function is dependent on the presence of both the EWS and FLI1 portion of the chimeric molecule. The data suggest two possible mechanisms by which chimeric transcription factors may contribute to tumor biology: (1) EWS-FLI1 may affect transcription of some of the same genes normally regulated by native FLI1, but because the chimeric molecule is expressed in a different temporal or quantitative manner, the downstream targets are deregulated, affecting the growth of cells; and (2) EWS-FLI1 may affect target genes different from those regulated by native FLI1, which would explain why overexpression of FLI1 does not have the same transforming properties as overexpression of EWS-FLI1.[59] These two mechanisms are not mutually exclusive.

It is likely that the ability of these EWS-ETS chimeric proteins to modulate transcription of specific genes is associated with their oncogenic potential. The critical target genes contributing to tumor biology are not known, but analysis of RNAs differentially expressed between stably FLI1- and EWS-FLI1–transfected NIH3T3 cells resulted in the identification of several interesting genes.[60] Some of these genes are implicated in control of cell growth, differentiation, and oncogenesis. *MFNG* is a member of the Fringe gene family encoding secreted signaling molecules instrumental in somatic development.[61,62] Although ectopic *MFNG* expression does not completely recapitulate the effects of EWS-FLI1 overexpression, it can render NIH3T3 cells tumorigenic in immunodeficient mice.[63] Stromelysin is a metaloproteinase that could contribute to the ability of tumor cells to invade the surrounding connective tissue. *EAT2* (EWS-FLI1–activated transcript 2) has features of a signal transduction molecule.[64] *E2-C* is a cyclin-specific ubiquitin-conjugating enzyme and has been related to cell-cycle control and cyclin B degradation.[65] Other genes that may be affected by EWS-FLI1 include *MYCC*, a transcription factor directly related to cell proliferation and apoptosis that is apparently upregulated in an indirect manner, and genes harboring a serum response element, such as the oncogene c-*fos* or *egrl*. This is based on the observation that EWS-FLI1 is able to bind to the serum response element and form a complex with the serum response factor.[56,66,67] It is expected that the target genes deregulated by EWS-FLI1 must be central to ES/PNET biology. Their identification is key to further understanding of this disease.

Cell Transformation

The consistent presence of these chimeric molecules in the ES/PNET family suggests a critical role in tumor biology. In experiments to evaluate the ability of fusion proteins to cause neoplastic transformation, the chimeric protein has been shown to affect the growth characteristics of some cell lines. Transfection of *EWS-FLI1* or *EWS-ERG* can transform mouse NIH3T3 fibroblasts so that they acquire tumor-like properties, such as growth in soft agar and immunodeficient mice.[54,59] Both the EWS and FLI1 domains are required for transforming activity. Transfection of wild-type *FLI1* does not result in cell transformation, which suggests that *EWS-FLI1* is functionally distinct. Not all cell types are efficiently transformed by *EWS-FLI1*, which suggests that chimeric molecule function is dependent on a permissive cellular background. This may indicate that other cellular proteins modulate the function of EWS-FLI1. In this regard, it has been shown that EWS-FLI1 is able to cooperate with other transcription factors to activate a model reporter gene and that expression of insulin-like growth factor 1 receptor is required for NIH3T3 transformation.[68,69] Cell context may, therefore, have an important bearing on the role of tumor-specific chromosomal translocations, as described above.

The fusion protein has also been shown to be important for maintaining the growth characteristics of ES/PNET cell lines. EWS-FLI1 RNA antisense molecules transfected into ES/PNET cells decrease expression of EWS-FLI1 and result in growth inhibition in culture and tumor xenografts.[70,71] In addition, truncated ETS domain–binding molecules can act as competitive inhibitors and have a dominant negative effect on cell growth.[72] This implies that a certain level of expression of the chimeric molecule and its downstream targets is necessary for the growth-related affects of EWS-FLI1 and suggests a promising field of investigation for novel therapeutic approaches.

EWS-FLI1 may also exert tumorigenic effects via deregulation of programmed cell death. Cancer is believed to result in part from an imbalance in cell growth and cell death. Expression of EWS-FLI1 or EWS-ERG in NIH3T3 cells inhibits apoptosis that would normally occur with serum deprivation or calcium ionophore treatment.[73] In addition, antisense inhibition of EWS-FLI1 results in increased susceptibility to chemotherapy-induced apoptosis in

ES/PNET cell lines. These data suggest that chimeric molecules may affect mechanisms of programmed cell death and thereby play a role not only in tumor development but also in response to therapy.

MOLECULAR DIAGNOSTICS

Routine techniques in surgical pathology allow the diagnosis of most cases of ES and PNET, but genetic analysis is very helpful when morphology is not conclusive or tumors present in unusual clinical settings.[74-76] Standard cytogenetic studies can reveal a wide variety of chromosomal alterations, but technical constraints and variant translocations may make interpretation difficult, and results are often obtained too late to influence therapy. Molecular assays for specific chromosomal changes, such as those found in ES and PNET, are attractive for several reasons. Two clinically important features are the small sample size requirement and the ability to provide results rapidly. Such studies are also considered more sensitive than traditional cytogenetics, with a higher technical success rate for detecting specific gene fusions and the potential to detect cryptic translocations. A particularly appealing advantage for diagnostic molecular studies is that, despite distortions of histologic patterns or the absence of histologic or immunohistochemical evidence of differentiation, the basic molecular genetic alterations are present in tumor cells and molecular analysis may permit greater confidence in a specific diagnosis.

Many molecular techniques have proven useful for detection of the EWS translocations associated with ES and PNET, including fluorescent in situ hybridization, Southern blotting, DNA-based polymerase chain reaction (PCR), and RNA-based PCR (also referred to as reverse transcriptase [RT]-PCR).[77] The translocations associated with ES and PNET can be readily identified using RT-PCR by the presence of fusion transcripts in tumor cells, and this technique has become a mainstay in molecular diagnosis. RT-PCR is extremely specific and sensitive and allows detection of very low levels of tumor cells even when they present among large numbers of normal cells, such as circulating tumor cells in peripheral blood or bone marrow. This provides an extremely robust method for molecular staging and monitoring treatment response. Stage of disease at diagnosis as determined by standard imaging modalities constitutes the most powerful predictor of prognosis for patients with ES or PNET. Some patients considered to have localized disease, however, may have unfavorable outcome due to minimal metastatic disease not detected by traditional methods. Several groups have demonstrated that minimal disease can be detected in peripheral blood and bone marrow using PCR-based methods and may contribute to patient management. Nevertheless, a number of technical, biologic, and clinical questions remain.[78-82]

Molecular Markers of Prognosis

In ES and PNET, several factors have been considered to be of prognostic importance (stage, primary tumor site, size, age, and response to therapy).[10] Recent studies have evaluated the contribution of molecular heterogeneity in the ES/PNET family to prognosis.[50,83-85] Gene fusions in ES and PNET show molecular variability, with at least 18 different structural possibilities. There are two sources of variability: the fusion partner (*FLI1*, *ERG*, *ETV1*, *E1A*, or *FEV*) and the breakpoint location within the genes. A better outcome for patients with localized tumors expressing the most common chimeric transcript (*EWS* exon 7 fused to *FLI1* exon 6) compared with other fusion types has been reported. This raises the possibility that heterogeneity in chimeric transcript structure may reliably define clinically distinct risk groups. The biologic basis for the prognostic difference between EWS fusion types is unknown, but a recent report has shown functional differences among the various fusion genes.[86] Direct comparison between treatment response and *EWS* fusion type may reveal a role of certain chimeras in therapy resistance. These reports should stimulate inclusion of *EWS* fusion-type determination into prospective studies.

Studies evaluating the prognostic significance of other cytogenetic and molecular alterations in ES/PNET are limited. However, del1p, homozygous deletion of *CDKN2A*, and *TP53* mutation have all been associated with a worse prognosis.[50] The association of genetic variants of ES and PNET with clinically significant features is an area that deserves further study.

Therapeutic Potential of Molecular Targets

Identification of molecular characteristics of the ES/PNET family has contributed to clinical care by providing a greater degree of confidence for an often difficult diagnostic problem. An equally exciting area of current investigation is the exploitation of these tumor-specific biologic properties as targets for novel therapeutic approaches. Current efforts have focused on immunotherapy directed at tumor cell–specific epitopes derived from chimeric molecules, but approaches to directly inhibit chimeric proteins and their downstream targets also seem feasible.[87] Further understanding of the basic biology of these tumor-specific molecules has the potential to lead to novel therapies.

In summary, identification and characterization of the *EWS-FLI1* fusion in the ES/PNET family of tumors further support the oncogenic role of transcription-modulating chimeric proteins resulting from chromosomal translocations.

The elucidation of specific pathogenetic contributions of fusion genes will give insight into the development and maintenance of the malignant phenotype for this family of tumors. Of practical importance, further investigation of the structural and functional attributes of the *EWS-FLI1* gene fusion has begun to provide the necessary basic information to directly benefit individuals with ES and PNET.

REFERENCES

1. Stout AP: A tumor of the ulnar nerve. Proc NY Pathol Soc 12:2-12, 1918
2. Ewing J: Diffuse endothelioma of bone. Proc NY Pathol Soc 21:17-24, 1921
3. Askin FB, Rosai J, Sibley RK, et al: Malignant small cell tumor of the thoracopulmonary region in childhood: A distinctive clinicopathologic entity of uncertain histogenesis. Cancer 43:2438-2451, 1979
4. Tefft M, Vawter GF, Mitus A: Paravertebral "round cell" tumors in children. Radiology 92:1501-1509, 1969
5. Llombart-Bosch A, Lacombe MJ, Contesso G, et al: Small round blue cell sarcoma of bone mimicking atypical Ewing's sarcoma with neuroectodermal features: An analysis of five cases with immunohistochemical and electron microscopic support. Cancer 60:1570-1582, 1987
6. Jaffe R, Santamaria M, Yunis EJ, et al: The neuroectodermal tumor of bone. Am J Surg Pathol 8:885-898, 1984
7. Angervall L, Enzinger FM: Extraskeletal neoplasm resembling Ewing's sarcoma. Cancer 36:240-251, 1975
8. Kovar H: Ewing's sarcoma and peripheral primitive neuroectodermal tumors after their genetic union. Curr Opin Oncol 10:334-342, 1998
9. Zucman-Rossi J, Batzer M, Stoneking M, et al. Interethnic polymorphism of EWS intron 6: Genome plasticity mediated by Alu retroposition and recombination. Hum Genet 99:357-363, 1997
10. Terrier P, Llombart-Bosch A, Contesso G: Small round blue cell tumors in bone: Prognostic factors correlated to Ewing's sarcoma and neuroectodermal tumors. Semin Diagn Pathol 13:250-257, 1996
11. Dehner L: Primitive neuroectodermal tumor and Ewing's sarcoma. Am J Surg Pathol 17:1-13, 1993
12. Fellinger E, Garin-Chesa P, Triche TJ, et al: Immunohistochemical analysis of Ewing's sarcoma cell surface antigen p30/32^{MIC2}. Am J Pathol 139:317-326, 1991
13. Ambros I, Ambros P, Strehl S, et al: MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors. Cancer 67:1886-1893, 1991
14. Weidner N, Tjoe J: Immunohistochemical profile of monoclonal antibody O13: Antibody that recognizes glycoprotein p30/32^{MIC2} and is useful in diagnosing Ewing's sarcoma and peripheral neuroepithelioma. Am J Surg Pathol 18:486-494, 1994
15. Sandberg AA: The Chromosomes in Human Cancer and Leukemia. New York, NY, Elsevier Science Publishing Co, Inc, 1990
16. Aurias A, Rimbaut C, Buffe D, et al: Chromosomal translocations in Ewing's sarcoma. N Engl J Med 309:496-497, 1983
17. Whang-Peng J, Triche TJ, Knutsen T, et al: Chromosome translocation in peripheral neuroepithelioma. N Engl J Med 311:584-585, 1984
18. Delattre O, Zucman J, Plougastrel B, et al: Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumors. Nature 359:162-165, 1992
19. Zucman J, Delattre O, Desmaze C, et al: Cloning and characterization of the Ewing's sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. Genes Chromosom Cancer 5:271-277, 1992
20. Ohno T, Ouchida M, Lee L, et al: The EWS gene, involved in Ewing family of tumors, malignant melanoma of soft parts and desmoplastic small round cell tumors, codes for an RNA binding protein with novel regulatory domains. Oncogene 9:3087-3097, 1994
21. Rabbitts TH, Forster A, Larson R, et al: Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. Nat Genet 4:175-180, 1993
22. Crozat A, Aman P, Mandahl N, et al: Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 363:640-644, 1993
23. Zinszner H, Albalat R, Ron D: A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP. Genes Dev 8:2513-2526, 1994
24. Berlotti A, Lutz Y, Heard DJ, et al: hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. EMBO J 15:5022-5031, 1996
25. Hackl W, Lührmann R: Molecular cloning and subcellular localisation of the snRNP-associated protein 69KD, a structural homologue of the proto-oncoproteins TLS and EWS with RNA and DNA-binding properties. J Mol Biol 264:843-851, 1996
26. Alliegro MC, Alliegro MA: A nuclear protein regulated during the transition from active to quiescent phenotype in cultured endothelial cells. Dev Biol 174:288-297, 1996
27. Stolow DT, Haynes SR, Cabeza A: *Drosophila* gene encoding a novel RNA binding protein, shares homology with EWS and TLS, two genes involved in human sarcoma formation. Nucleic Acids Res 23:835-843, 1995
28. Immanuel D, Zinszner H, Ron D: Association of SARFH (sarcoma-associated RNA-binding fly homolog) with regions of chromatin transcribed by RNA polymerase II. Mol Cell Biol 15:4562-4571, 1995
29. Burd CG, Dreyfuss G: Conserved structures and diversity of functions of RNA-binding proteins. Science 265:615-621, 1994
30. Bertolotti A, Melot T, Acker J, et al: EWS, but not EWS-FLI-1, is associated with both TFIID and RNA polymerase II: Interactions between two members of the TET family, EWS and hTAFII68, and subunits of TFIID and RNA polymerase II complexes. Mol Cell Biol 18:1489-1497, 1998
31. Petermann R, Mossier BM, Aryee DN, et al: Oncogenic EWS-Fli1 interacts with hsRPB7, a subunit of human RNA polymerase II. Oncogene 17:603-610, 1998
32. Hromas R, Klemsz M: The ETS oncogene family in development, proliferation and neoplasia. Int J Hematol 59:257-265, 1994
33. Sharrocks AD, Brown AL, Ling Y, et al: The ETS-domain transcription factor family. Int J Biochem Cell Biol 29:1371-1387, 1997
34. Dittmer J, Nordheim A: Ets transcription factors and human disease. Biochim Biophys Acta 1377:F1-11, 1998
35. Wasylyk B, Hahn SL, Giovane A: The Ets family of transcription factors. Eur J Biochem 211:7-18, 1993

36. Zucman J, Melot T, Desmaze C, et al: Combinatorial generation of variable fusion proteins in the Ewing family of tumors. EMBO J 12:4481-4487, 1993

37. Sorensen PHB, Lessnick SL, Lopez-Terrada D, et al: A second Ewing's sarcoma translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. Nat Genet 6:146-151, 1994

38. Jeon I-S, Davis JN, Braun BS, et al: A variant Ewing's sarcoma translocation t(7;22) fuses the EWS gene to the ETS gene ETV1. Oncogene 10:1229-1239, 1995

39. Urano F, Umezawa A, Hong W, et al: A novel chimera gene between EWS and E1A-F, encoding the adenovirus E1A enhancer-binding protein, in extraosseous Ewing's sarcoma. Biochem Biophys Res Commun 219:608-612, 1996

40. Peter M, Couturier J, Pacquement H, et al: A new member of the ETS family fused to EWS in Ewing tumors. Oncogene 14:1159-1164, 1997

41. Wasylyk B, Hagman J, Gutierrez-Hartmann A: Ets transcription factors: Nuclear effectors of the Ras-MAP-kinase signaling pathway. Trends Biochem Sci 23:213-216, 1998

42. Zucman J, Delattre O, Desmaze C, et al: EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nat Genet 4:341-345, 1993

43. Ladanyi M, Gerald WL: Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumor. Cancer Res 54:2837-2840, 1994

44. Panagopoulos I, Hoglund M, Mertens F, et al: Fusion of EWS and CHOP genes in myxoid liposarcoma. Oncogene 12:489-494, 1996

45. Labelle Y, Zucman F, Stenman G, et al: Oncogenic conversion of a novel orphan nuclear receptor by chromosome translocation. Hum Mol Genet 4:2219-2226, 1995

46. Clark J, Benjamin H, Gill S, et al: Fusion of the EWS gene to CHN, a member of the steroid/thyroid receptor gene superfamily, in a human myxoid chondrosarcoma. Oncogene 12:229-235, 1996

47. Panagopoulos I, Aman P, Fioretos T, et al: Fusion of the FUS gene with ERG in acute myeloid leukemia with t(16;21)(p11;q22). Genes Chromosom Cancer 11:256-262, 1994

48. Ichikawa H, Shimizu K, Hayashi Y, et al: An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. Cancer Res 54:2865-2868, 1994

49. Armengol G, Tarkkanen M, Virolainen M, et al: Recurrent gains of 1q, 8 and 12 in the Ewing family of tumours by comparative genomic hybridization. Br J Cancer 75:1403-1409, 1997

50. Hattinger CM, Rumpler S, Strehl S, et al: Prognostic impact of deletions at 1p36 and numerical aberrations in ES/PNET. Genes Chromosom Cancer 24:243-254, 1999

51. Kovar H, Auinger A, Jug G, et al: Narrow spectrum of infrequent p53 mutations and absence of MDM2 amplification in Ewing tumours. Oncogene 8:2683-2690, 1993

52. Hamelin R, Zucman J, Melot T, et al: P53 mutations in human tumors with chimeric EWS/FLI-1 genes. Int J Cancer 57:336-340, 1994

53. Kovar H, Jug G, Aryee DN, et al: Among genes involved in the RB dependent cell cycle regulatory cascade, the p16 tumor suppressor gene is frequently lost in the Ewing family of tumors. Oncogene 15:2225-2232, 1997

54. May WA, Lessnick SL, Braun BS, et al: The Ewing's sarcoma EWS/FLI-1 fusion gene encodes a more potent transcriptional activator and is a more powerful transforming gene than FLI-1. Mol Cell Biol 13:7393-7398, 1993

55. Ohno T, Rao VN, Reddy ESP: EWS/Fli-1 chimeric protein is a transcriptional activator. Cancer Res 53:5859-5863, 1994

56. Bailly RA, Bosselut R, Zucman J, et al: DNA-binding and transcriptional activation properties of the EWS-FLI-1 fusion protein resulting from the t(11;22) translocation in Ewing sarcoma. Mol Cell Biol 14:3230-3241, 1994

57. Mao X, Miesfeldt S, Yang H, et al: The FLI-1 and chimeric EWS-FLI-1 oncoproteins display similar DNA binding specificities. J Biol Chem 269:18216-18222, 1994

58. Zhang D, Paley A, Childs G: The transcriptional repressor ZFM1 interacts with and modulates the ability of EWS to activate transcription. J Biol Chem 273:18086-18091, 1998

59. May WA, Gishizky ML, Lessnick SL, et al: Ewing sarcoma 11;22 translocation produces a chimeric transcription factor that requires the DNA-binding domain encoded by FLI1 for transformation. Proc Natl Acad Sci U S A 90:5752-5756, 1993

60. Braun BS, Frieden R, Lessnick SL, et al: Identification of target genes for the Ewing's sarcoma EWS/Fli fusion protein by representational difference analysis. Mol Cell Biol 15:4623-4630, 1995

61. Johnston SH, Rauskolb C, Wilson R, et al: A family of mammalian Fringe genes implicated in boundary determination and the Notch pathway. Development 124:2245-2254, 1997

62. Yuan YP, Schultz J, Mlodzik M, et al: Secreted fringe-like signaling molecules may be glycosyltransferases. Cell 88:9-11, 1997

63. May WA, Arvand A, Thompson AD, et al: EWS-FLI1-induced manic fringe renders NIH 3T3 cells tumorigenic. Nat Genet 17:495-497, 1997

64. Thompson AD, Braun BS, Arvand A, et al: EAT-2 is a novel SH2 domain containing protein that is up regulated by Ewing's sarcoma EWS-FLI1 fusion gene. Oncogene 13:2649-2658, 1996

65. Arvand A, Bastians H, Welford SM, et al: EWS-FLI1 up regulates mE2-C, a cyclin-selective ubiquitin conjugating enzyme involved in cyclin B destruction. Oncogene 17:2039-2045, 1998

66. Magnaghi-Jaulin L, Masutani H, Robin P, et al: SRE elements are binding sites for the fusion protein EWS-FLI-1. Nucleic Acids Res 24:1052-1058, 1996

67. Watson DK, Robinson L, Hodge DR, et al: FLI1 and EWS-FLI1 function as ternary complex factors and ELK1 and SAP1a function as ternary and quaternary complex factors on the Egr1 promoter serum response elements. Oncogene 14:213-221, 1997

68. Lessnick SL, Braun BS, Denny CT, et al: Multiple domains mediate transformation by the Ewing's sarcoma EWS/FLI-1 fusion gene. Oncogene 10:423-431, 1995

69. Toretsky JA, Kalebic T, Blakesley V, et al: The insulin-like growth factor-I receptor is required for EWS/FLI-1 transformation of fibroblasts. J Biol Chem 272:30822-30827, 1997

70. Tanaka K, Iwakuma T, Harimaya K, et al: EWS-Fli1 antisense oligodeoxynucleotide inhibits proliferation of human Ewing's sarcoma and primitive neuroectodermal tumor cells. J Clin Invest 99:239-247, 1997

71. Toretsky JA, Connell Y, Neckers L, et al: Inhibition of EWS-FLI-1 fusion protein with antisense oligodeoxynucleotides. J Neurooncol 31:9-16, 1997

72. Kovar H, Aryee DN, Jug G, et al: EWS-FLI1 antagonists induce growth inhibition of Ewing tumor cells in vitro. Cell Growth Differ 7:429-437, 1996

73. Yi H, Fujimura Y, Ouchida M, et al: Inhibition of apoptosis by normal and aberrant Fli-1 and erg proteins involved in human solid tumors and leukemias. Oncogene 14:1259-1268, 1997

74. Delattre O, Zucman J, Melot T, et al: The Ewing family of tumors: A subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med 331:294-299, 1994

75. de Alava E, Ladanyi M, Rosai J, et al: Detection of chimeric transcripts in desmoplastic small round cell tumor and related developmental tumors by reverse transcriptase polymerase chain reaction: A specific diagnostic assay. Am J Pathol 147:1584-1591, 1995

76. Barr FG, Chatten J, D'Cruz CM, et al: Molecular assays for chromosomal translocations in the diagnosis of pediatric soft tissue sarcomas. JAMA 273:553-557, 1995

77. Ladanyi M: The emerging molecular genetics of sarcoma translocations. Diagn Mol Pathol 4:162-173, 1995

78. Pfleiderer C, Zoubek A, Gruber B, et al: Detection of tumour cells in peripheral blood and bone marrow from Ewing tumour patients by RT-PCR. Int J Cancer 21:135-139, 1995

79. West DC, Grier HE, Swallow MM, et al: Detection of circulating tumor cells in patients with Ewing's sarcoma and peripheral primitive neuroectodermal tumor. J Clin Oncol 15:583-588, 1997

80. de Alava E, Lozano MD, Patino A, et al: Ewing family tumors: Potential prognostic value of reverse-transcriptase polymerase chain reaction detection of minimal residual disease in peripheral blood samples. Diagn Mol Pathol 7:152-157, 1998

81. Fagnou C, Michon J, Peter M, et al: Presence of tumor cells in bone marrow but not in blood is associated with adverse prognosis in patients with Ewing's tumor. J Clin Oncol 16:1707-1711, 1998

82. Zoubek A, Ladenstein R, Windhager R: Predictive potential of testing for bone marrow involvement in Ewing tumor patients by RT-PCR: A preliminary evaluation. Int J Cancer 79:56-60, 1998

83. Zoubek A, Pfleiderer C, Salzer-Kuntschik M, et al: Variability of EWS chimaeric transcripts in Ewing tumours: A comparison of clinical and molecular data. Br J Cancer 70:908-913, 1994

84. Zoubek A, Dockhorn-Dworniczak B, Delattre O, et al: Does expression of different EWS chimeric transcripts define clinically distinct risk groups of Ewing tumor patients? J Clin Oncol 14:1245-1251, 1996

85. de Alava E, Kawai A, Healey JH, et al: EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. J Clin Oncol 16:1248-1255, 1998

86. Lin PP, Brody RI, Hamelin A, et al: Differential transactivation by alternative EWS-FLI1 fusion proteins correlates with clinical heterogeneity in Ewing's sarcoma. Cancer Res 59:1428-1432, 1999

87. Goletz TJ, Mackall CL, Berzofsky JA, et al: Molecular alterations in pediatric sarcomas: Potential targets for immunotherapy. Sarcoma 2:77-87, 1998